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THE MULTIELEMENT ANALYSIS OF BIOLOGICAL MATERIAL BY NEUTRON ACTIVATION AND DIRECT INSTRUMENTAL TECHNIQUES (a)

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INTRODUCTION

Although several studies concerning the trace element concentrations in biological organisms have been conducted over the years [1], the roles of most trace elements in biological processes remain poorly understood. This situation can, in part, be attributed to the lack of suitable analytical technology which will permit precise multielement analysis in the parts-per-million to sub-parts-per-billion range. Two analytical techniques, emission spectroscopy [2], and spark source mass spectrometry [3], have been shown to be capable of simultaneous multielement analysis of biological material. However, these methods require extensive sample preparation with its associated contamination problems, and are seriously limited in sensitivity for many trace constituents by matrix effects and spectral interferences. The extension of trace element measurements to permit an analysis of the fine structure of the body organs and of cellular areas within the organs requires even

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DISCLAIMER

greater care in sample aquisition, sample preparation and analysis together with a very sensitive analytical method.

A great deal can be learned regarding the metabolic role of a trace element having a known biological function. Also, from the large elemental variations which have been observed within an organism [4], it is evident that the concentration of the intra-related trace elements should be simultaneously measured.

Neutron activation analysis has provided a practical means of solving many environmental and biomedical problems which require trace element sensitivities beyond the capability of other techniques. Previous work has been mainly concerned with the instrumental determination of a few specific elements or extensive multielement determinations involving radiochemical separations prior to gamma-ray analysis [5].

The objectives of this work have been to develop and apply comparative methods of neutron activation analysis which permit the direct instrumental measurement of some 20 trace elements in biological materials. These include a large number of the biologically important elements together with several elements which have unknown biological functions. The method involves a minimum of pre-irradiation handling, requiring only that the sample be freeze dried prior to neutron irradiation.

EXPERIMENTAL

Apparatus and reagents. Two irradiation facilities were employed in this investigation. The first, the heavy water moderated Plutonium Recycle Test Reactor (PRTR), has a rabbit system with a delivery time of about 10 seconds, and a neutron flux of about 2×10^{13} n/cm^2-sec with

a cadmium ratio in excess of 100. The second facility was a graphite moderated Hanford Production Reactor with it's characteristic high, well-thermalized neutron flux.

The gamma-ray spectrometric analysis of neutron activated samples was performed with two detector systems. The first consisted of a high resolution five-sided coaxial Ge(Li) detector with an active volume of 20 cc [6]. The second detector system was an anticoincidence shielded, gamma-gamma coincidence multidimensional analyzer employing NaI(Tl) crystals [7].

Pre-irradiation sample preparations were performed in an Agnew-Higgins laminar flow work station. All biological samples were dissected with clean carbon steel scapels, placed directly in polyethylene capsules which had been cleaned with nitric acid, and dried in a Thermovac Industries Freeze Drier. This sampling procedure was shown to result in a negligible transfer of contamination to the sample [8].

Standard solutions were prepared from Mathy Spec Pure materials dissolved in re-distilled nitric acid and diluted with double distilled water. The neutron flux monitors consisted of high purity cellulose [9] on which known amounts of the standard solutions had been dried or 100 microliter aliquots of dilute (10^{-6} g/ml - 10^{-8} g/ml) solutions of each element of interest sealed in 3 mm I.D. quartz ampoules.

Procedure. The samples and standards were first irradiated in tandem in the rabbit facility of the PRTR reactor to an integrated thermal neutron exposure of 2×10^{15} n/cm^2. The polyethylene irradiation containers

were then vented to eliminate ^{41}Ar, and placed in a standard counting geometry above the Ge(Li) detector. Gamma-ray spectra of the activated samples and standards were obtained after appropriate decay intervals to determine the neutron induced activities of ^{24}Na, ^{27}Mg, ^{38}Cl, ^{42}K, ^{80}Br, and in the case of lung tissue, ^{28}Al. Typical gamma-ray spectra of neutron activated beef and fish muscle are presented in Figures 1 and 2.

Following a one week decay, the samples and a set of standards sealed in quartz ampoules were re-irradiated with an integrated thermal neutron exposure of 7×10^{17} n/cm^2. After a decay interval of two days, the samples were pulverized, suspended in a 2% agar solution (contained in 1/2" x 2" rings to provide a standard counting geometry) and counted on a Ge(Li) spectrometer along with appropriate standards to permit the determination of Na, K and Br from their neutron activation products ^{24}Na, ^{42}K, and ^{82}Br. After a decay interval of about twenty days, a second Ge(Li) gamma-ray spectrum revealed the characteristic photopeaks of ^{51}Cr, ^{59}Fe, ^{60}Co, ^{65}Zn, ^{75}Se, ^{86}Rb, ^{134}Cs, and ^{203}Hg. In the case of some human lung tissue samples, ^{46}Sc, ^{124}Sb, and ^{233}Pa were also observed, and in spectra taken about 10 to 15 days after irradiation, ^{198}Au and ^{239}Np were present. The initial identification of each photopeak was based on measurements of energy, relative spectral intensity and half-life. It was necessary to strip the component of ^{75}Se from the 279 keV photopeak of ^{203}Hg prior to calculating the concentration of mercury. All other photopeaks employed in the analysis were found to be free of spectral interferences.

A third set of gamma-ray spectra of the sample and standards was obtained with the gamma-gamma coincidence multidimensional analyzer about

45 days after irradiation and from these, the parent elements of the observed radionuclides ^{46}Sc, ^{60}Co, ^{65}Zn, ^{110m}Ag, ^{124}Sb, and ^{134}Cs, were determined.

RESULTS AND DISCUSSION

Results. The accuracy and precision of this instrumental method of analysis was estimated by analyzing high purity cellulose samples to which known amounts of each element of interest had been added. The average measured concentrations of trace elements in three samples of this material and the standard deviation of the average [10] are compared with actual concentrations in Table 1. The observed average concentrations compare quite well with the actual concentrations except for Ag and Sc. The agreement is within the errors associated with counting statistics plus the estimated experimental error associated with sample self-shielding, contamination, flux-gradients and interfering nuclear reactions [8].

Several types of biological material, high purity cellulose air filters, human lung tissue, beef muscle, and fish muscle are being analyzed by this technique in a variety of environmental and biological programs and a few examples which demonstrate the applicability of the method are included. The results of the analyses of some of these materials are presented in Table 2 together with the standard deviation of each average where replicate analyses were performed and the total estimated experimental error.

The value of the simultaneous measurement of the concentrations of several elements in a single sample of tissue is demonstrated from our study of the variation of cesium and rubidium in an organism relative to potassium. The ratios of rubidium and cesium to potassium in various types of muscle tissue of a silver salmon are presented in Table 3 together with the ratios reported in sea water. This observation suggests that while rubidium and potassium are taken up by this organism in direct proportion to their concentration in sea water, the organism has the ability to differentiate and accumulate cesium relative to potassium. Concentration factors of 3 to 8 in the cesium to potassium ratio of the organism relative to the ratio in sea water are not explained by simple diffusion since the hydrated ionic radius of Cs, Rb and K are quite similar [11].

Discussion. The direct instrumental neutron activation analysis of biological material employing both Ge(Li) gamma-ray spectrometry and NaI(Tl) gamma-gamma coincidence multidimensional spectrometry has been shown to be a sensitive, accurate and precise method of trace element analysis. The versatility of the method permits the determination of some 20 elements in a single sample of dry tissue without resorting to chemical separations. The method is currently being employed to study a variety of environmental and biomedical problems including the trace element distribution through the organs and muscle of terrestrial, aquatic and marine organisms and man.

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LIST OF FIGURES

Figure 1. Gamma Spectra of Neutron Activated Beef Muscle

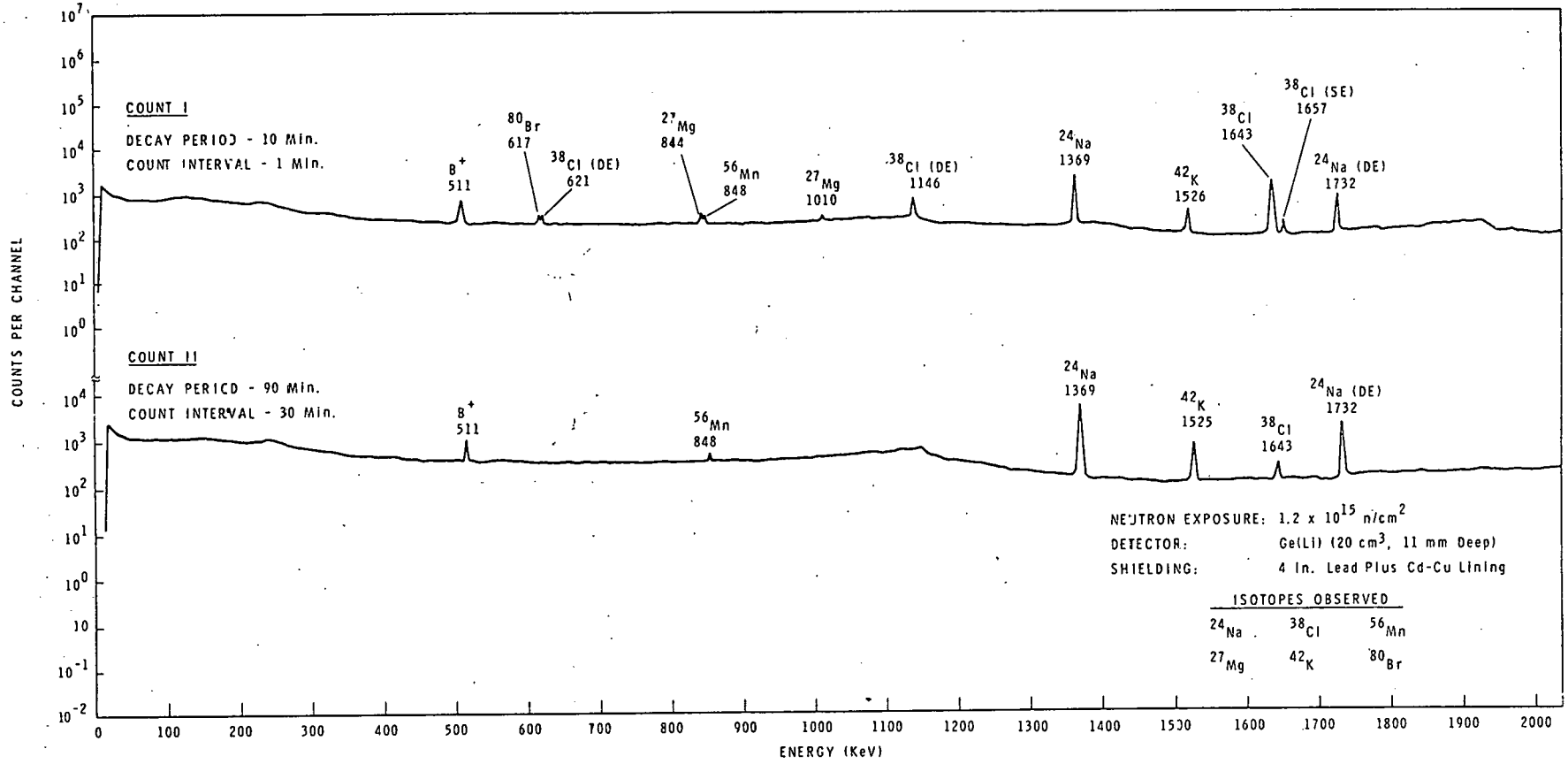
Figure 2. Gamma Spectra of Neutron Activated Fish Muscle

LIST OF TABLES

Table 1. Results of the Analysis of Synthetic Biological Samples by Neutron Activation

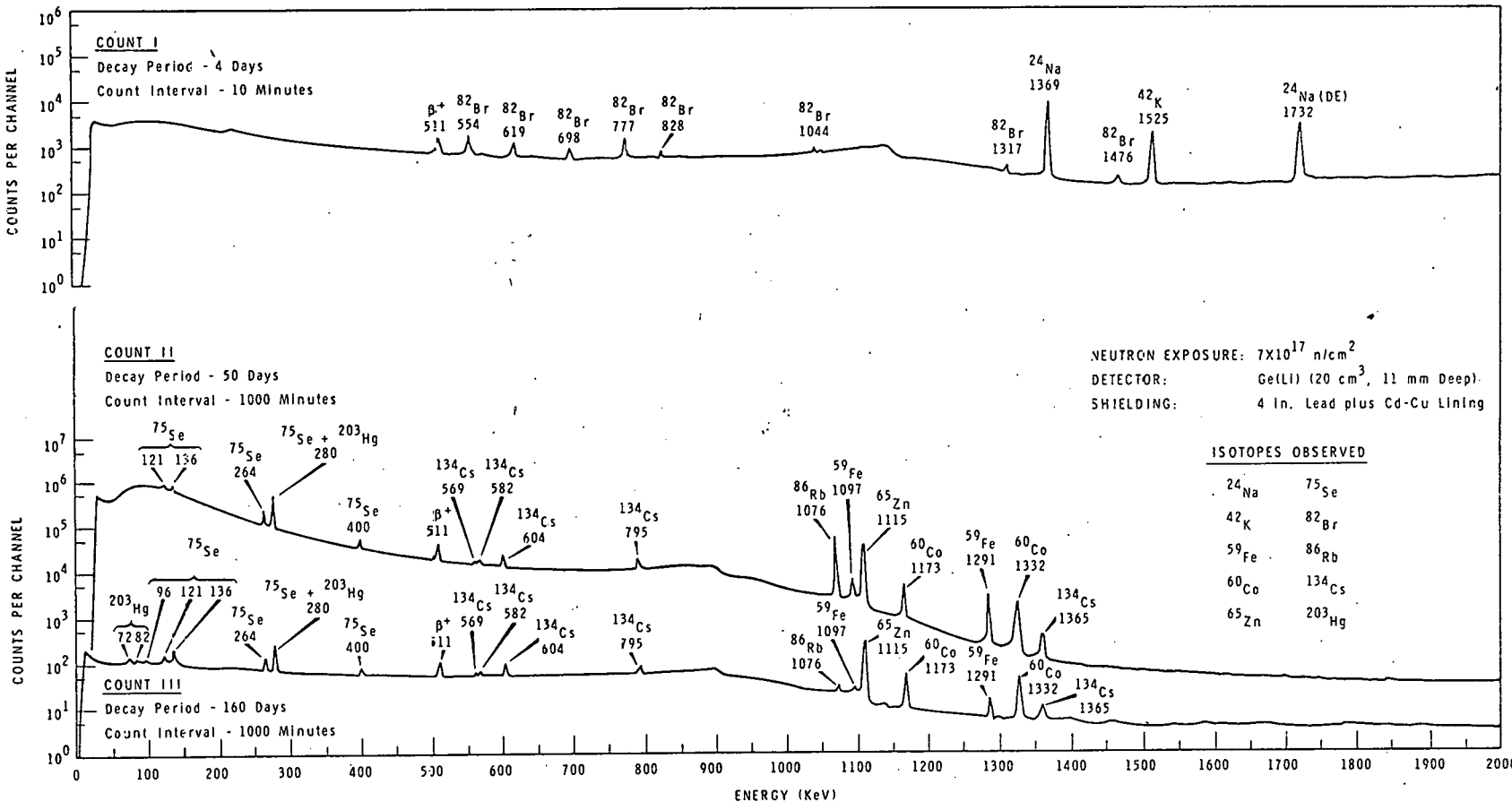
Table 2. Trace Element Concentrations of Biological Materials as Determined by Neutron Activation Analysis

Table 3. Relative Concentrations of Cesium, Potassium, and Rubidium in Silver Salmon Tissue



GAMMA SPECTRA OF NEUTRON ACTIVATED BEEF MUSCLE

FIGURE 1



GAMMA SPECTRA OF NEUTRON ACTIVATED FISH MUSCLE

FIGURE 2

TABLE 1

RESULTS OF THE ANALYSIS OF SYNTHETIC BIOLOGICAL SAMPLES BY NEUTRON ACTIVATION

(Micrograms per gram of dry tissue)

Element	Actual Concentration	Average ± σ*	Estimated Experimental Error
Na	1700	1770 ± 60	5%
K	20,000	20,400 ± 700	5%
Rb	6.6	6.5 ± 0.6	5%
Cs	0.104	0.103 ± 0.006	5%
Br	10.8	10.8 ± 0.6	5%
Zn	83.4	87 ± 3	10%
Fe	33.2	33 ± 3	5%
Co	0.064	0.065 ± 0.006	10%
Se	0.44	0.41 ± 0.03	15%
Hg	0.52	0.49 ± 0.02	10%
Cr	1.2	1.3 ± 0.2	10%
Ag	0.014	0.012 ± 0.003	10%
Sb	0.026	0.027 ± 0.001	10%
Sc	0.010	0.012 ± 0.001	5%

*Average of three determinations.

TABLE 2

TRACE ELEMENT CONCENTRATIONS OF BIOLOGICAL MATERIALS AS DETERMINED BY NEUTRON ACTIVATION ANALYSIS

(micrograms per gram of dry tissue)

Element	IPC Filter (Cellulose)	Human Lung Tissue	Beef Muscle	Silver Salmon Muscle, Dorsal	Trout Muscle, Dorsal	Estimated Experimental Error %
K	<20	6300	11,400±200	16,800±100	18,900±1100	5
Cl	38±3	11,000	1430±60	---	---	5
Na	72±2	9000	1730±120	920±30	600±20	5
Mg	--	---	550±40	---	---	5
Fe	5.7±0.1	430	91±7	7.6±0.6	9.2±0.7	10
Al	4.6±0.4	190	<10	---	---	5-20
Zn	0.64±0.03	55	170±10	12±1	11.5±0.5	5
Br	0.16±0.02	15	11.9±0.9	26±2	10.5±0.5	5
Rb	---	10.4	4.2±0.2	5.6±0.1	16.6±0.1	5
Se	---	1.1	0.18±0.003	0.96±0.02	0.67±0.03	10
Cr	0.13±0.06	1.1	0.04±0.02	<0.02	0.12±0.05	5-15
Mn	1.2±0.1	---	0.31±0.02	---	---	5
Hg	---	0.14	<0.10	0.30±0.05	0.39±0.01	15
Co	0.0026±0.0001	0.092	0.014±0.001	0.0055±0.0005	0.011±0.0005	5
Cs	0.0003±0.00004	0.041	0.010±0.001	0.105±0.005	0.110±0.004	5
Sb	0.0015±0.0002	0.070	0.0006±0.0001	0.0001	0.0004±0.0002	5-20
Au	---	0.040	---	---	---	10
Ag	<0.001	0.019	<0.001	0.001	0.0014±0.0002	5-20
Sc	0.00040±0.00001	0.021	0.00056±0.00007	0.00003±0.00001	0.00004±0.00001	5-50
Number of Analysis	5	1	3	2	2	

TABLE 3

RELATIVE CONCENTRATIONS OF CESIUM, POTASSIUM, AND RUBIDIUM IN SILVER SALMON TISSUE

	Relative Concentration	
	Rb/K ($\times 10^4$)	Cs/K ($\times 10^6$)
Red Muscle	3.6 ± 0.2	6.8 ± 0.1
White Muscle	3.4 ± 0.1	6.2 ± 0.3
Cheek Muscle	4.0 ± 0.3	5.8 ± 0.2
Liver	3.8 ± 0.6	2.5 ± 0.5
Sea Water [12, 13]	3.3	0.8